Source data files Weberruss et al.
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Figure 4B.

SDS-PAGE and Coomassie blue staining of Blm10C (lane 1), wt-GST-Gsp1 (lane 2),

GST-Gsp1-Q71L (lane 3) and GST (lane 4).

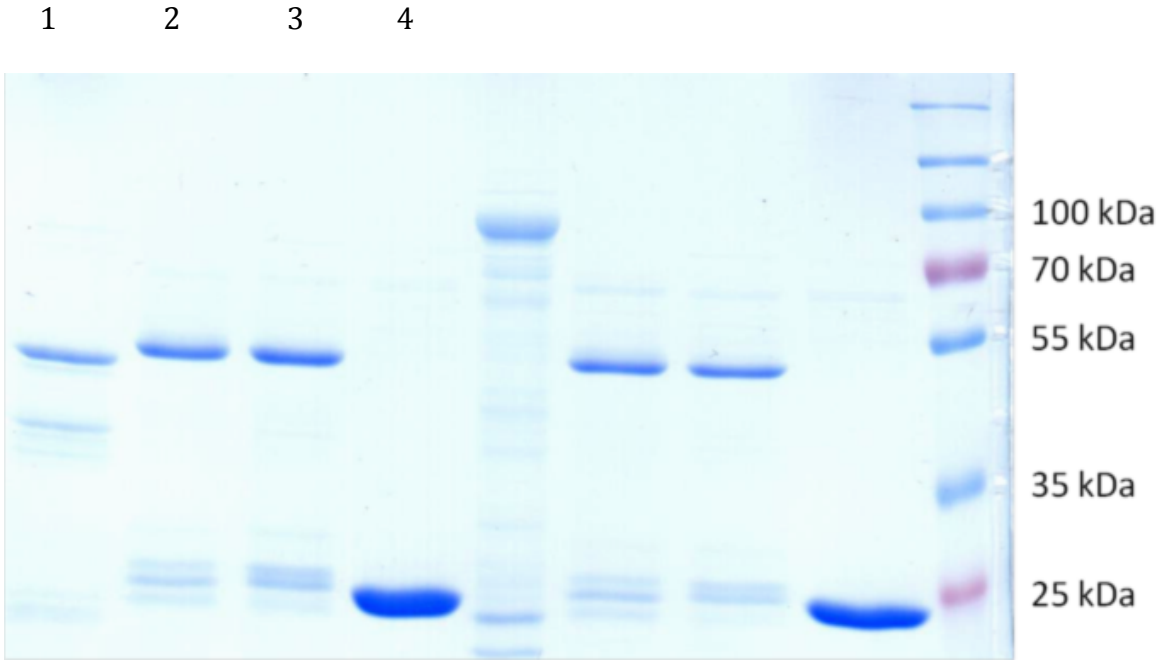

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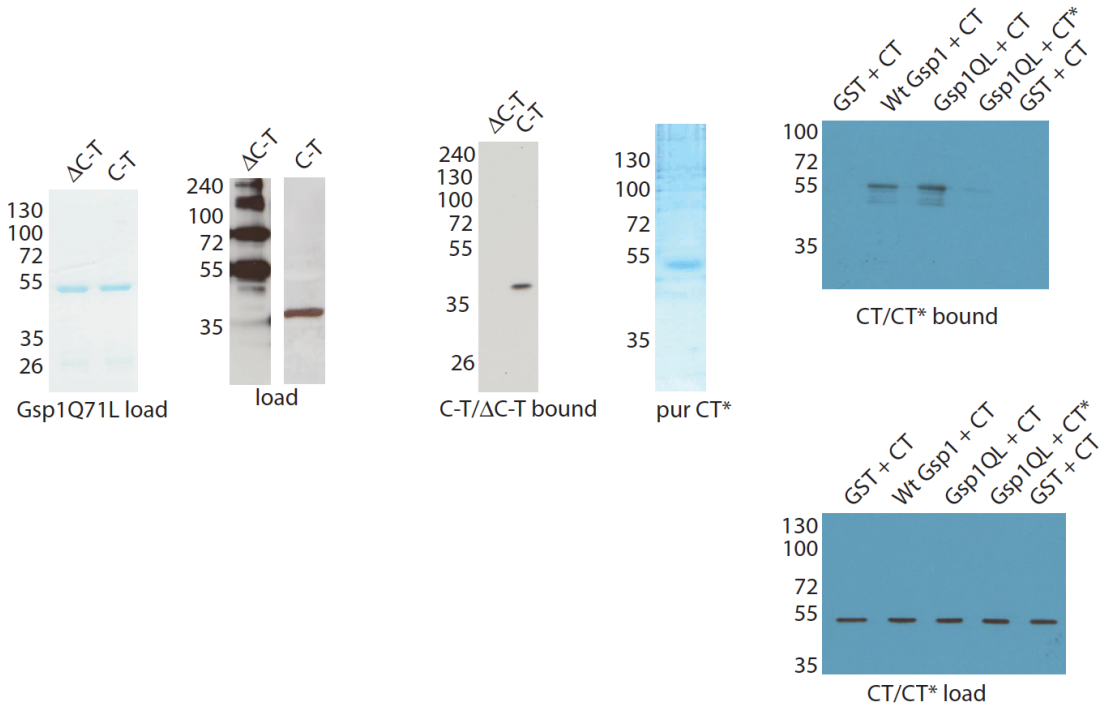
Figure 4C.

The minimized panels were taken from the following full size gels. ΔC-T is equivalent to Blm10ΔC1804-2143. C-T is equivalent to Blm10-C1804-2143. CT* is equivalent to Blm10C-W2021A. From the left to the right are shown:

Load of GST-Gsp1Q71L. Load of C-terminally truncated Blm10ΔC1804-2143 which is highly sensitive to degradation and load of Blm10C-1804-2143 labeled with ΔC-T and CT, respectively. Bound fraction of Blm10ΔC and Blm10C labeled with ΔC-T and CT, respectively (see Fig. 3C, lanes 1 and 2). Purified Blm10C is shown in the gel above, lane 1 (corresponds to Fig. 3C, lane 3). Purified Blm10C-W2021 is named pur CT* (corresponds to Fig. 3C, lane 4).



Lanes 1, 3 and 4 of the load and bound fractions of the right panels were excised and cropped for lanes 5 to 7 of Fig. 3C.

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Figure 4D.

The minimized panels were taken from the following full size gels.

GST-Nup53 migrates as a fuzzy band.

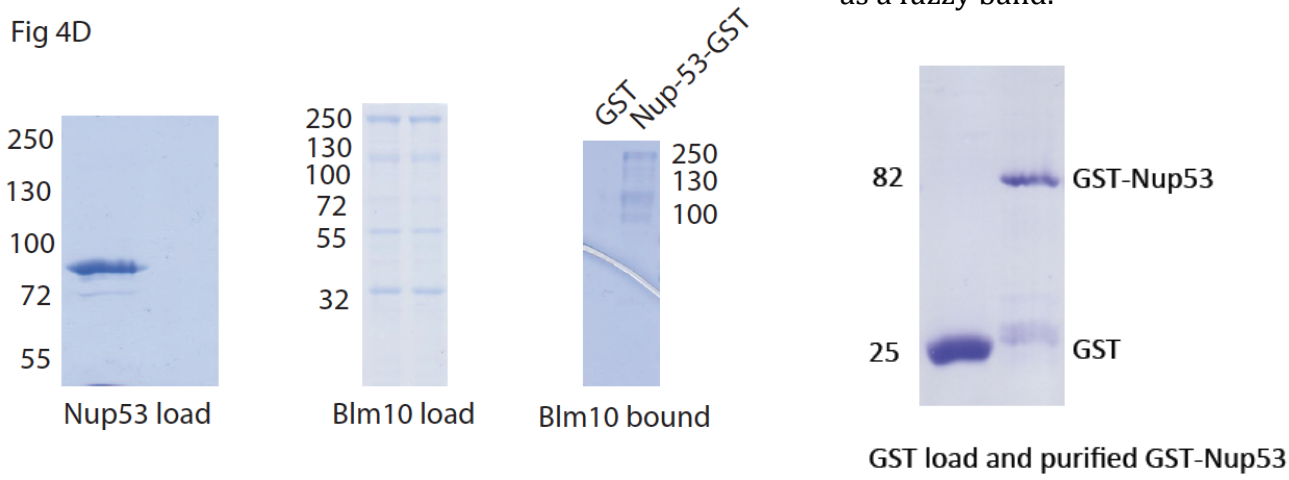

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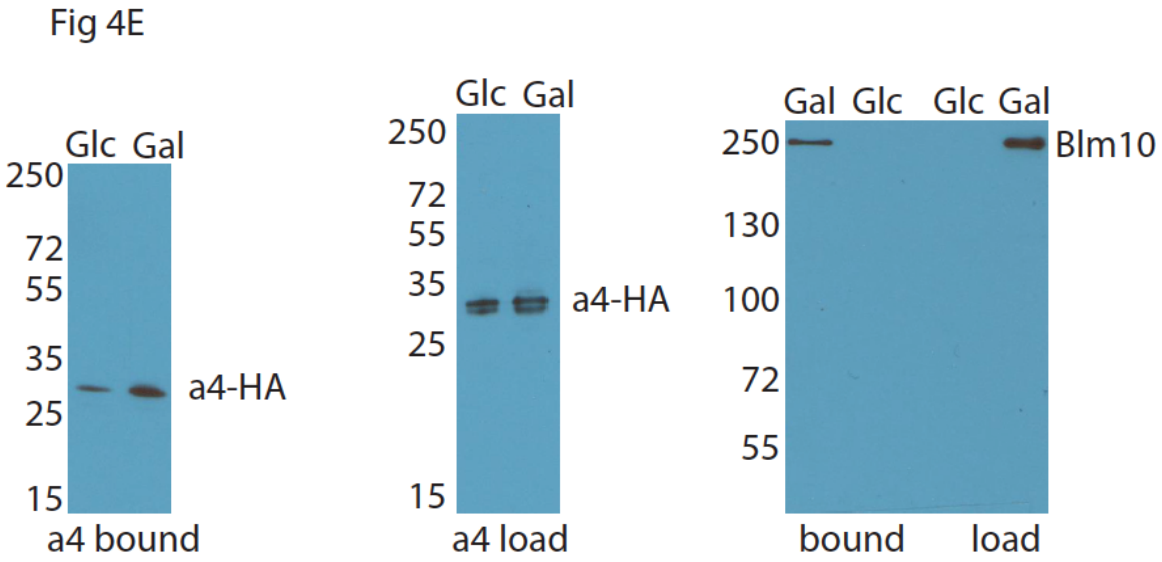
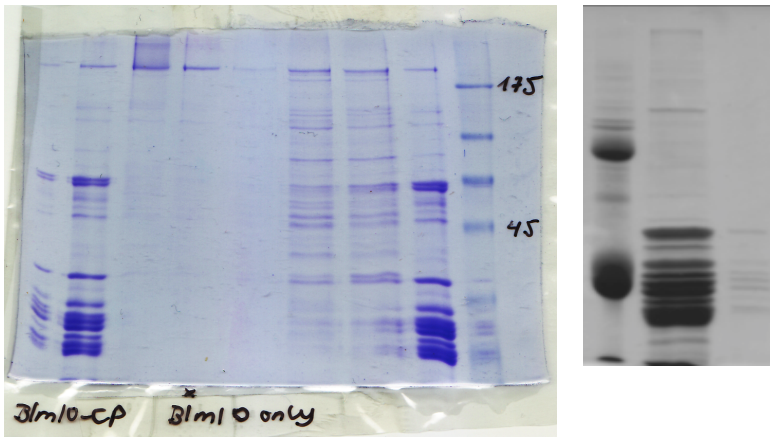
Figure 4E.

The minimized panels were taken from the following full size gels.



Load of purified Blm10 (x only) and CP were excised from the following images (lower left gel). Purifications of yeast CP are routinely performed in the lab (lower grey panel).



SDS-PAGE and Coomassie blue staining.

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Figure 4F.

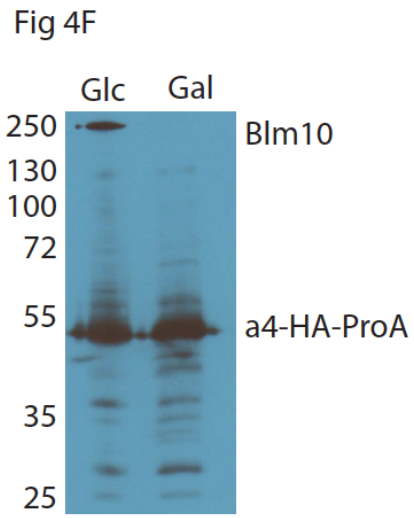
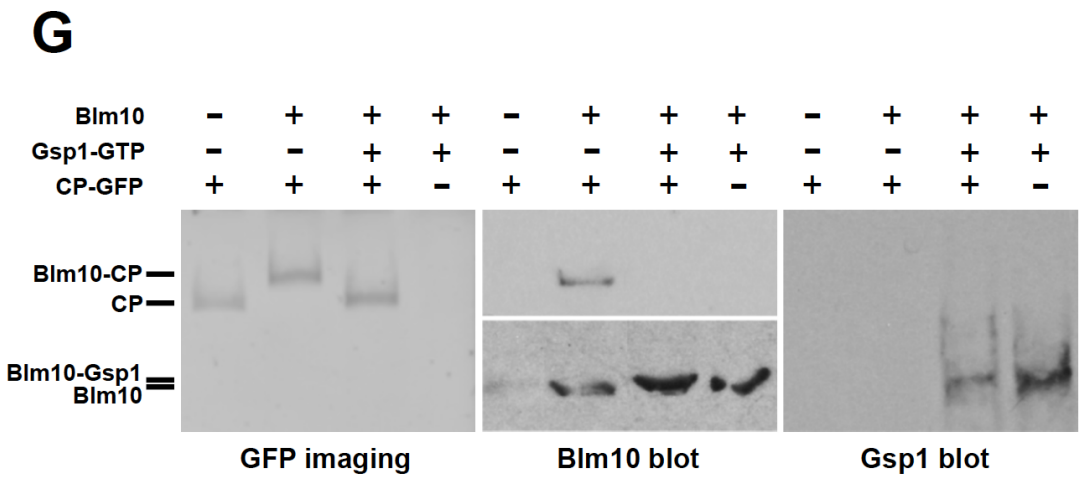


Figure 4G.

The experiment was repeated six times. Fig. 4G shows a representative experiment. The GFP image of the native PAGE gel is shown in full size (left panel). The gel is semi-dry blotted for 20 min at 400 mA on PVDF membrane.

Immunoblot with anti-Gsp1 / Ran antibodies (1:1,000 dilution) is shown in full size (right panel).



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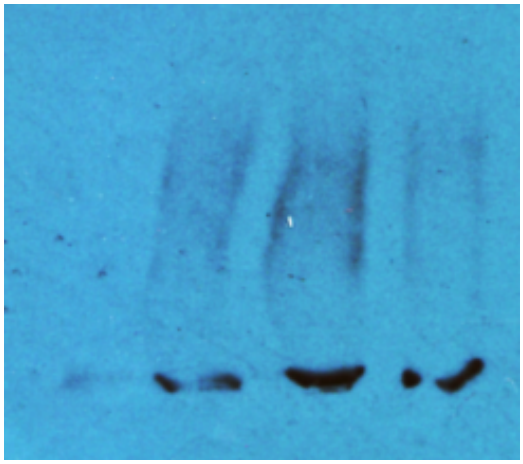
The gel shift in native PAGE towards a slower migrating species is indicative for the association of Blm10 and CP to Blm10-CP. The gel shift towards a faster migrating species is indicative for the dissociation of Blm10-CP into Blm10 and CP.

Immunoblot detection of His-tagged Blm10 in native PAGE gels is technically challenging.

Immunoblot detection using anti-Blm10 antibodies (1,000 dilution) reveals free Blm10 better than Blm10-bound CP. The lower part was excised from the blot and shown in Fig. 4F (middle panel).

original anti-Blm10 blot



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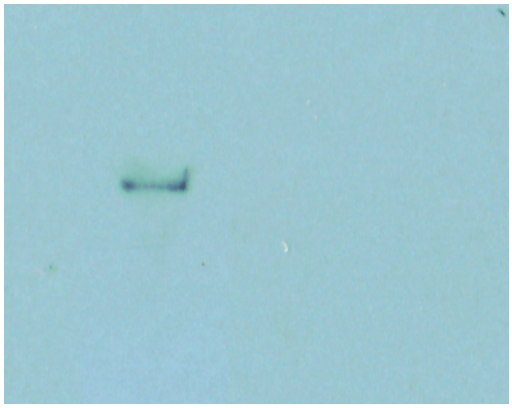
original anti-His tag blot



His-tagged Blm10 is highly sensitive to degradation and must be used immediately after purification. Full length His-tagged Blm10 bound to the CP was detected by anti-His tag antibodies (mouse IgG 6AT18 Abgent). For unknown reasons the fast migrating free Blm10 is hardly recognized by anti-His tag antibodies suggesting that the His-tag is cleaved off upon dissociation.

The upper part of the blot was excised and shown in Fig. 4F (middle panel).